# LABORATORY NOTES

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## A container for the transport of mounted crystals

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### Abstract

An airtight crystal-shipping tube has been produced by modification of a 15 ml pressure reaction tube to hold a mounted crystal. A crystal mounted on a specimen pin is held in place in the crystal-shipping tube by a set screw. The crystal-shipping tubes are then inserted in holes prepared for them in two foam rubber blocks. The foam rubber blocks fit into a Styrofoam box, and the whole package is shipped by overnight courier. Both the foam rubber packing and the fact that the specimen pin is firmly held in place minimize the mechanical stresses that occur during transport. Furthermore, the airtight container eliminates any pressure changes that may occur. In addition, the mounted crystals are visible through the glass shipping tube. A number of macromolecular data sets have been successfully collected from crystals shipped in this way.


### 1. Introduction

We often grow crystals at one site and then transfer them to another site for data collection. Sometimes this represents only a short walk down the hall or drive to a neighboring diffraction facility. With the increasing use of synchrotron light sources, however, this has meant transferring crystals by airplane, either by personal delivery or by overnight courier. Secure methods have been developed to transport cryo-crystals in liquid nitrogen (Rodgers, 1996).

Data from many crystals have gone uncollected, and many trips have been wasted, due to damage sustained by the crystals during transport. When they are flown from one destination to another, crystals are subjected to a number of changes. First, the atmospheric pressure in flight drops about $0.2 \times 10^5$ Pa to approximately $0.8 \times 10^5$ Pa. Second, there can be temperature fluctuations during the trip. Finally, the crystals can be jostled. The last two disturbances can be avoided by personal carrying of the crystals on an airplane. Significant expense and time could be saved if crystals could be shipped by overnight courier.

Below, we describe the procedures and shipping containers that we have successfully used to ship crystals by overnight courier from Richmond, California, USA to: San Diego, California, USA; The Woodlands, Texas, USA; and Berlin, Germany.

### 2. Crystal mounting

Prior to shipping, we mount the crystals. This minimizes the handling of the crystals and ensures that a crystal of interest has been successfully mounted prior to departure. Crystals cannot be shipped in most apparatus in which they are grown. A notable exception is the crystallization apparatus used in space (Holemans *et al.*, 1991). An alternative is to transfer the crystal to a shipping container at the crystallization site, ship the crystal, and then mount the crystal at the data collection site. We prefer not to use this procedure because the crystal is handled twice.

Crystals are mounted as follows. Two capillary tubes are selected: a glass capillary (The Charles Supper Company, Natick, MA, USA) sufficiently large to withdraw the crystal from the crystallization drop and a quartz capillary (The Charles Supper Company) sufficiently large to accommodate the first capillary within it. For example, if the maximum dimension of a crystal is 0.3 mm, then we use a 0.5 mm glass capillary and a 0.7 mm quartz capillary. The sealed end of the quartz capillary is left intact and is glued into a brass specimen pin (The Charles Supper Company). In the past, we have also used wax to mount the capillary in the specimen pin. The crystal is picked up from the crystallization drop using the glass capillary and deposited in the mounted quartz capillary about 1 to 2 cm above the specimen pin. The excess mother liquor is wicked away with a thin piece of filter paper. The saturated filter-paper wick is partially withdrawn, cut to 1 to 2 cm in length and then pushed back into the mounted capillary so that it is about 1 cm above the crystal. Finally, just above the wick, the top 1 to 2 cm of the capillary is filled with immersion oil (Hampton Research, Riverside, CA, USA). We prefer sealing

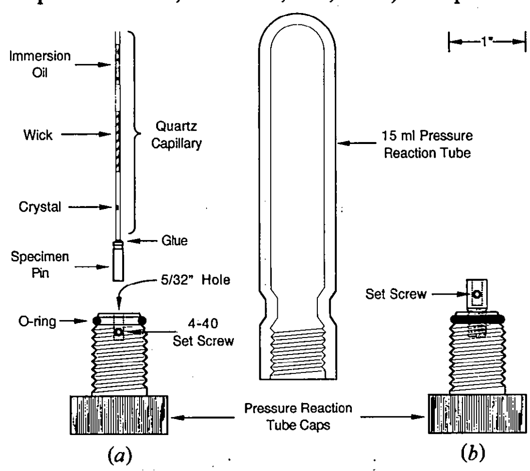


Fig. 1. Schematic diagram of the crystal-shipping tube and a mounted crystal. (*a*) The crystal-shipping-tube cap with the set screw below the O ring (current design). (*b*) A proposed design of the crystal-shipping-tube cap using an aluminum plug. The glass top of the crystal-shipping tube is shown between the two caps.

the capillary with immersion oil, for it makes a better seal than modeling clay, and prevents the heating of the crystal that occurs when the capillary is sealed with melted wax.

## 3. Shipping containers

Three points were considered in our search for, and design of, a mounted-crystal shipping container. First, we wanted the container to be airtight, so that the pressure changes during flight would not affect the crystal by pushing out the oil seal at the end of the mounted capillary. Second, we wanted the crystal to be firmly held in place, so that if the package was inverted, the crystal in its capillary mount would survive intact. Finally, we wanted a well padded shipping container to insulate the crystal from mechanical insult.

These design criteria were met by an airtight tube that firmly held the crystal in a foam-filled box. An airtight 15 ml pressure reaction tube (Alltech, Deerfield, IL, USA) was modified to hold a crystal specimen pin as follows: A 5/32 in (4 mm) hole is drilled approximately 3/8 in (9.5 mm) deep into the center of the Teflon screw cap of a pressure reaction tube. A second 1/16 in (1.6 mm) hole is drilled just below the O ring of the Teflon cap to intersect with the 5/32 in hole (see Fig. 1). A 4-40 × 5/8 in (16 mm) hex set screw is screwed into the 1/16 in hole, such that when a sample specimen pin holding a mounted crystal is placed into the 5/32 in hole, it can be held in place by the set screw.

Prior to the placing of a crystal into the modified pressure reaction tube, or crystal-shipping tube, as described above, the O ring and set screws are removed from the cap and all are covered with high-vacuum grease (Dow Corning Corporation, Midland, MI, USA). The greased O ring and set screw are then replaced. The mounted crystal on the specimen pin is placed in the 5/32 in hole and the set screw tightened until it is firmly in place. Note that over-tightening of the set screw can lead to scarring of the specimen pin. The cap of the crystal-shipping tube is then carefully screwed into the glass tube until the O ring has formed a seal. The seal can be monitored by observation of the level of the immersion oil in the capillary. The oil will move farther into the capillary as the seal is formed and the cap tightened.

In order to reduce mechanical insult to the crystal during transport, we wanted the crystal-shipping tube to be surrounded by foam rubber in a sturdy shipping box. Two 5 × 6 × 8 in (13 × 15 × 20 cm) pieces of foam rubber were cut from a 5 in (13 cm) thick piece of foam rubber (New York Fabrics, a local fabric store) such that they fitted into a 9 × 11.5 × 12.5 in (23 × 29 × 32 cm) FreezSafe® shipping box (Polyfoam Packers Corporation, Wheeling, IL, USA). These Styrofoam shipping boxes are easy to obtain, as they are used by a number of companies to ship biological samples. A 1 1/4 in (3 cm) diameter by 2 1/2 in (6 cm) deep hole was cut into each of the 5 in pieces of foam rubber to hold a crystal-shipping tube. The crystal-shipping tube is then inserted into the 1 1/4 in holes in the foam rubber cap-side down, and the second foam-rubber block is placed on top of the crystal-shipping tube. The Styrofoam shipping box and the foam rubber blocks provide sufficient insulation, such that a crystal could be kept cold by adding cold packs above and/or below the foam rubber in the taller 9 × 11.5 × 15.25 in (23 × 29 × 39 cm) FreezSafe® shipping box. A 4-40 hex wrench is placed in the shipping box, so that the person receiving the mounted crystal may easily free it from the cap of the crystal shipping tube. The sealed box is then shipped by overnight courier.

## 4. Results

Bovine trypsin crystals (Bode & Huber, 1978; Bartunik, Summers & Bartsch, 1989; Bode, Turk & Stürzebecher, 1990) were prepared using the hanging-drop vapor-diffusion technique at room temperature (Wlodawer & Hodgson, 1975) in Linbro culture plates (Gilliland & Davies, 1984). The crystal-shipping tubes described above have been used to successfully ship two such trypsin crystals to Molecular Structure Corporation in The Woodlands, Texas, USA, two to Area Detector Systems Corporation in San Diego, California, and one to Schering AG in Berlin, Germany. All of the data sets collected on rotating-anode generators diffracted to between 1.7 and 1.8 Å resolution. The data set collected on a sealed-tube generator diffracted to 2.1 Å.

## 5. Design improvements

A major improvement could be made in the design of the crystal-shipping tubes if the set screw could be put inside the O-ring seal. This would reduce or eliminate the need for high-vacuum grease, which is currently necessary to prevent air leaks through the set screw and into the crystal-shipping tube chamber through the specimen pin hole. In our newest design, a 1/8 in hole is drilled 3/8 in into a 1/4 in diameter by 3/4 in long cylindrical aluminum plug. A second 1/16 in hole is drilled 3/16 in into the side of the plug to intersect with the 1/8 in hole, and a 4-40 hex set screw is screwed into the 1/16 in hole. The bottom 3/8 in of the plug is threaded, and thus can be screwed into the Teflon screw cap of a pressure reaction tube, once a 1/4 in hole has been drilled and threaded 3/8 in deep into the center of the cap (see Fig. 1*b*).

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